

# FIGURE 1--Replacement of ERP with small molecule

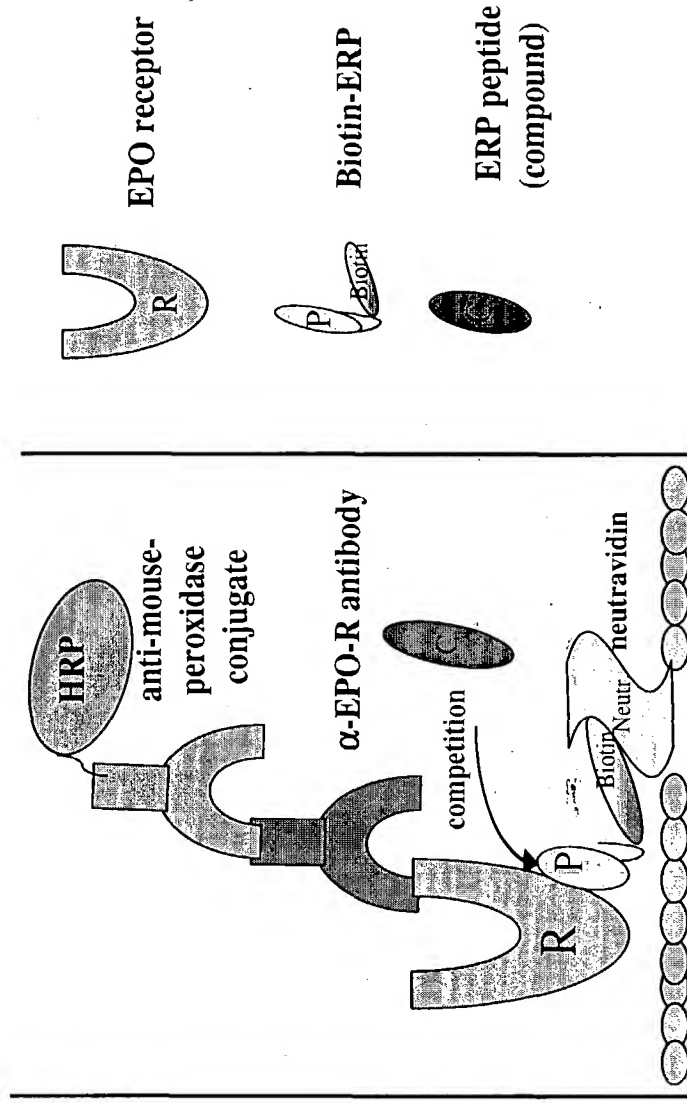
Primary screen

“peptide displacement assay”



Secondary screen

“functional assays”



# FIGURE 2--Role of small molecule in activation of EPO-R signaling pathway

## Study of small molecule effects and potential applications

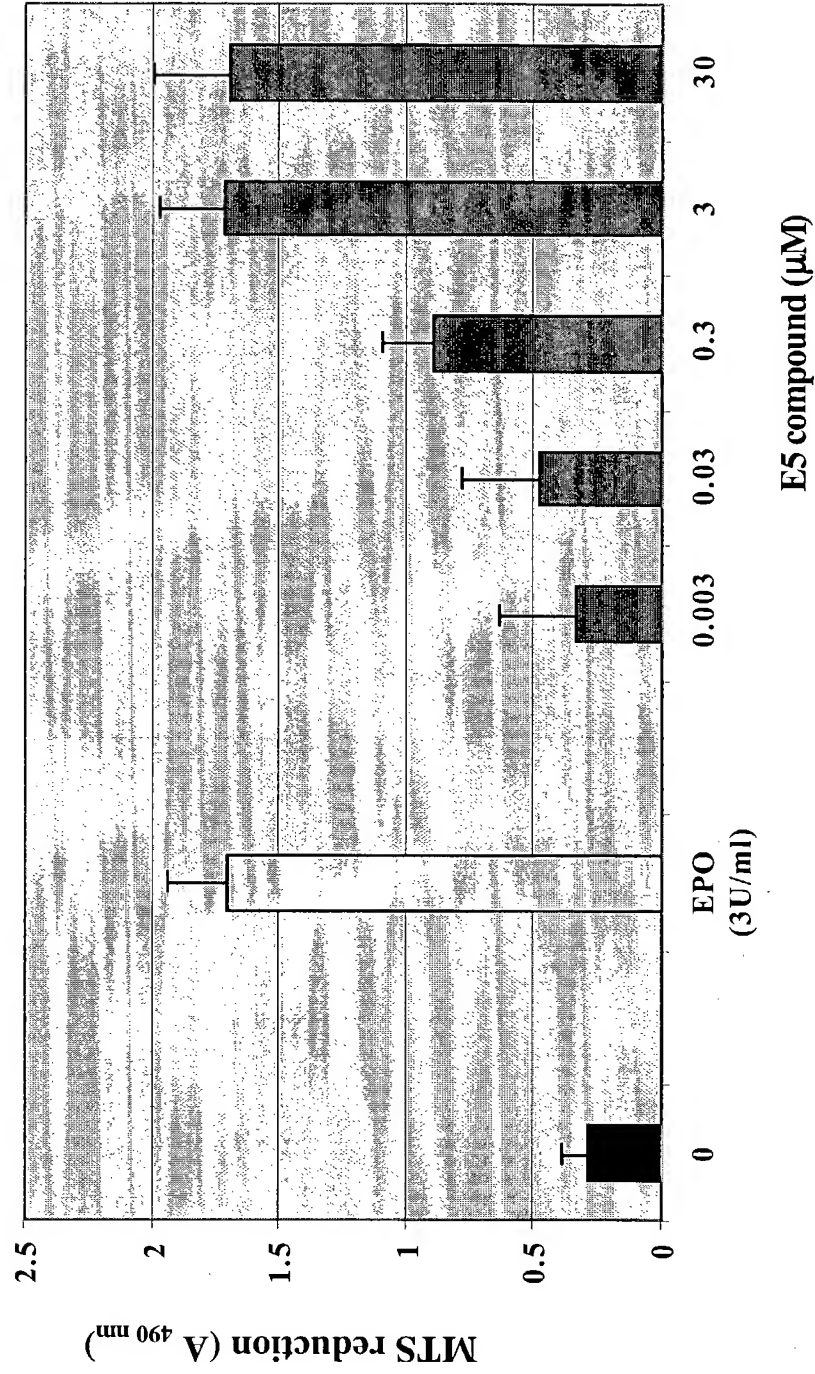
### *In vitro studies*

- Erythroid system
  - Activation of proliferative and anti-apoptotic pathway(s)
  - Colony formation in fetal liver cells
  - CFU-e/BFU-e formation in bone marrow (mouse and human)
- CNS system
  - Activation of anti-apoptotic pathway
  - Survival of neural-like cells upon serum withdrawal

### *In vivo studies*

- Effect in animals with carboplatin induced anemia; given I.p. and orally
- Reticulocytes levels in normal animals

FIGURE 3--Proliferative effect of E5 compound in  
TF-1 cells

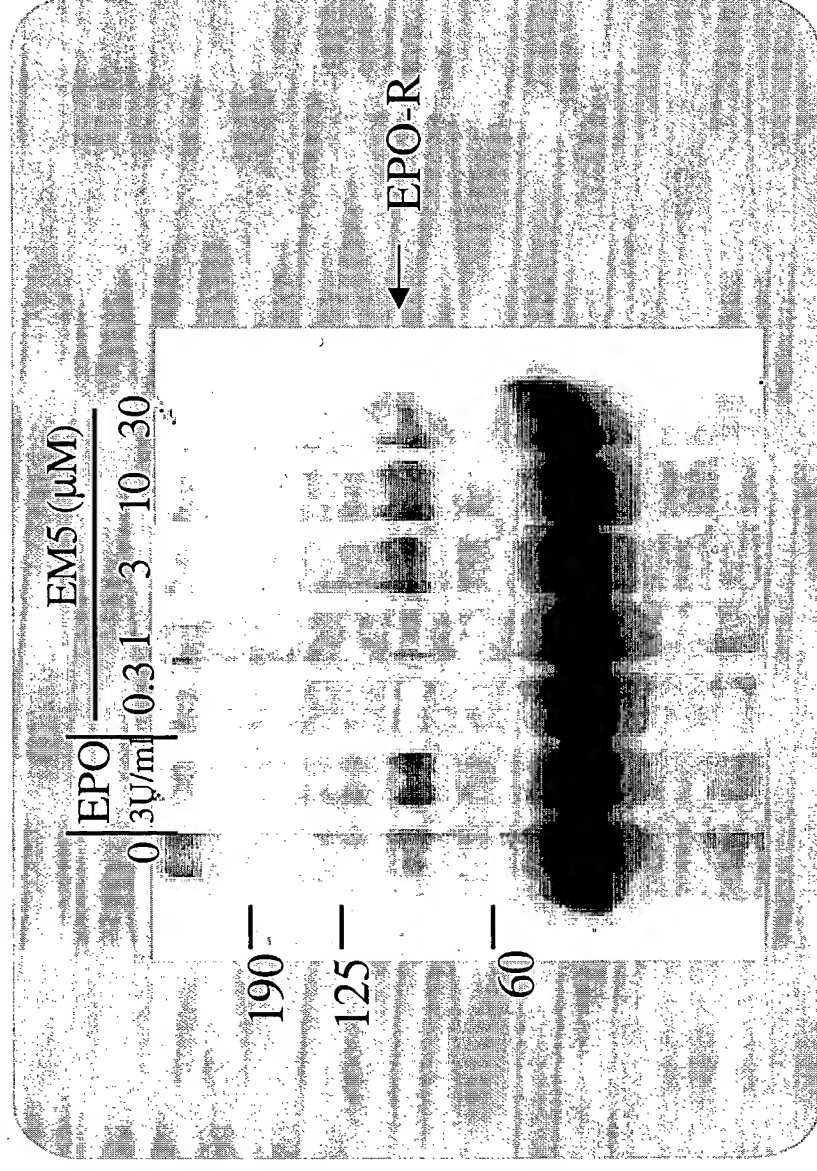


\* Same proliferative effect is observed with UT-7 cells

\* No effect in FDCP1 cells

# FIGURE 4--Activation of signaling cascade through EPO-R by small molecule

Small molecules bind to and activate/phosphorylate EPO-R (UT-7 cells)



IP: α-EPO-R Ab (Upstate technology)  
WB: α-PY Ab (Upstate technology)

FIGURE 5--Small molecules promote colony  
formation in the presence of SCF  
Fetal liver cells (day 3)

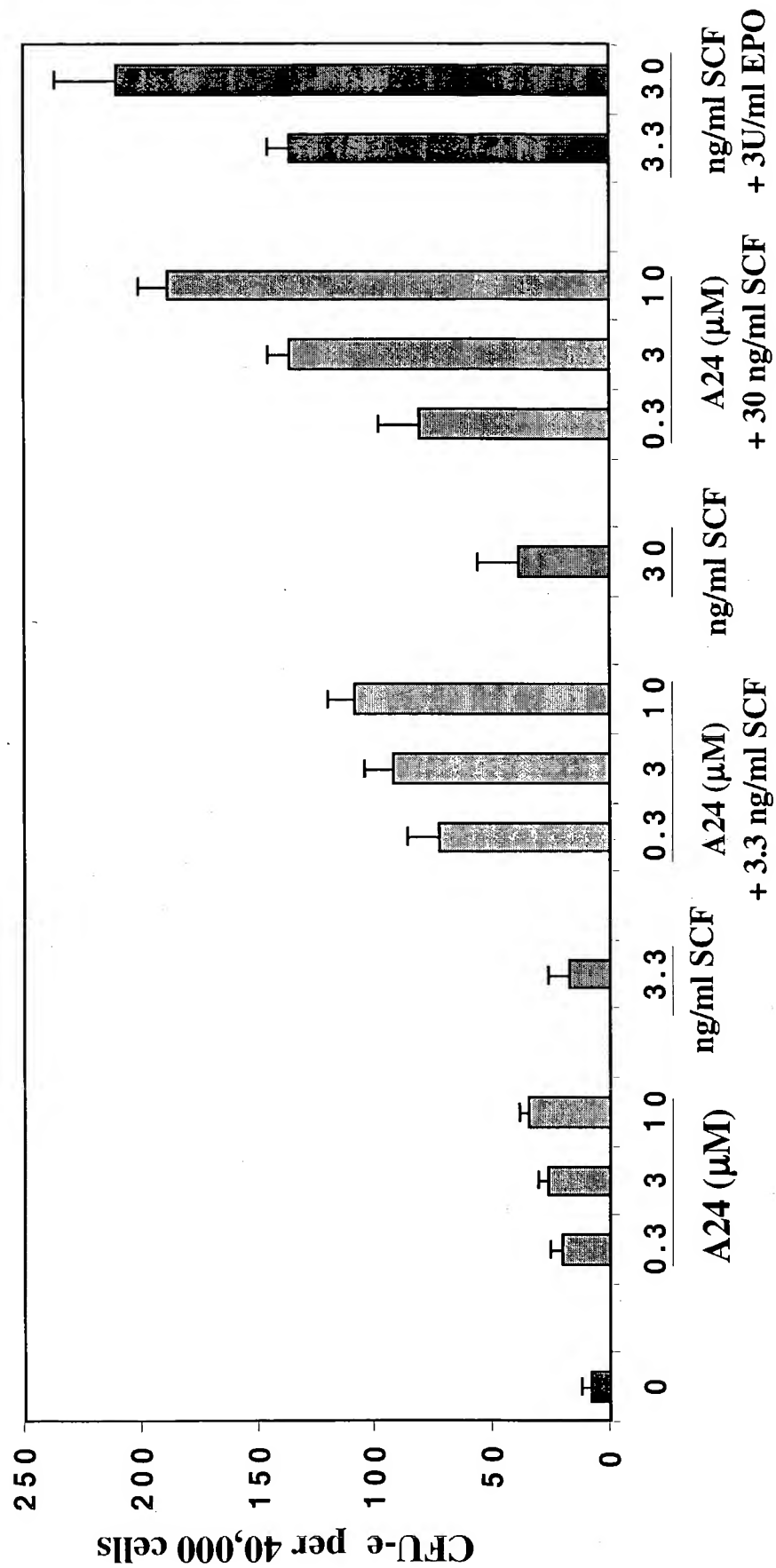


FIGURE 6--Effect of EPO-like small molecule on  
erythroid colony formation in methylcellulose  
Human bone marrow

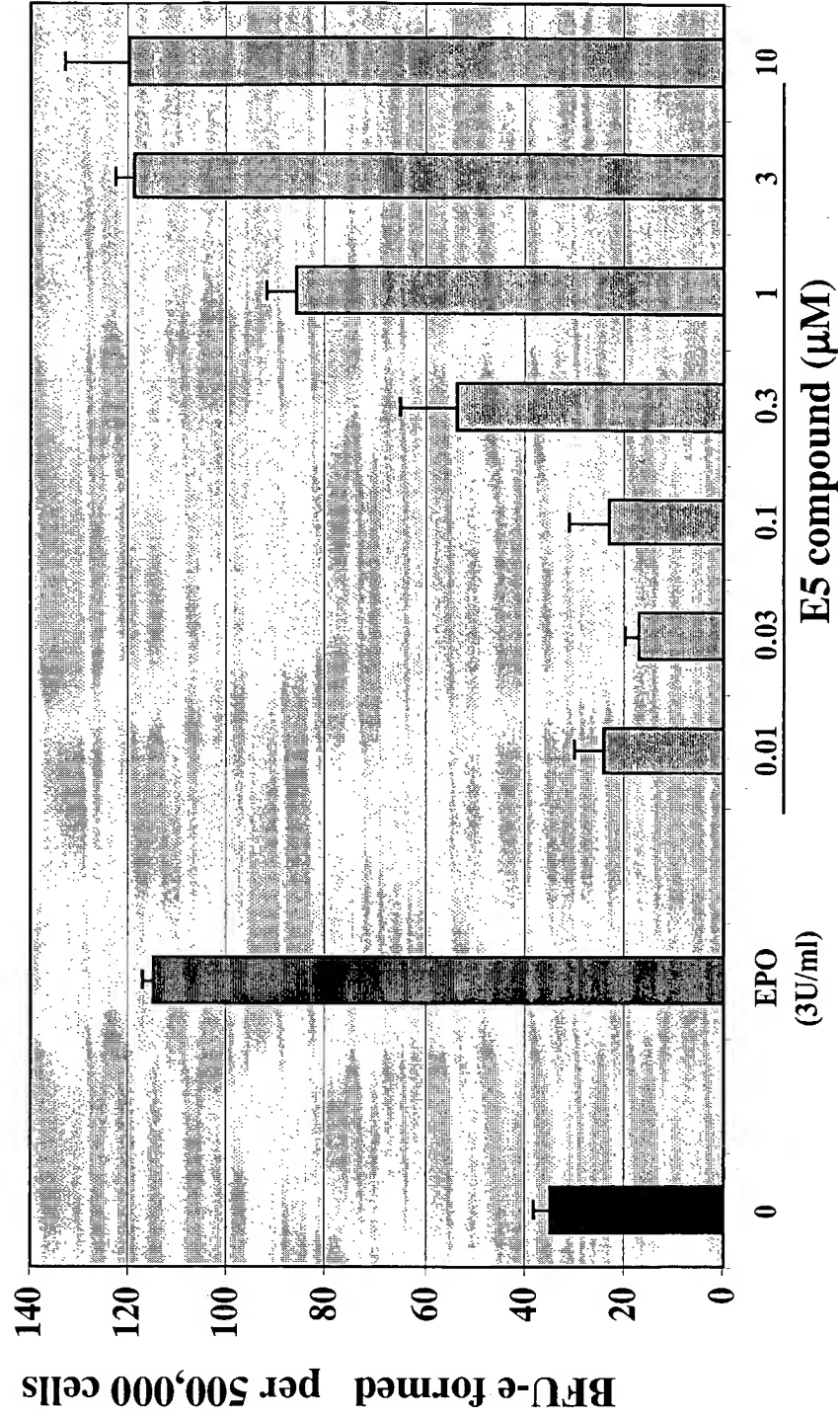


FIGURE 7-- Synergy between EM5 small molecule  
and EPO on erythroid colony formation  
Human bone marrow

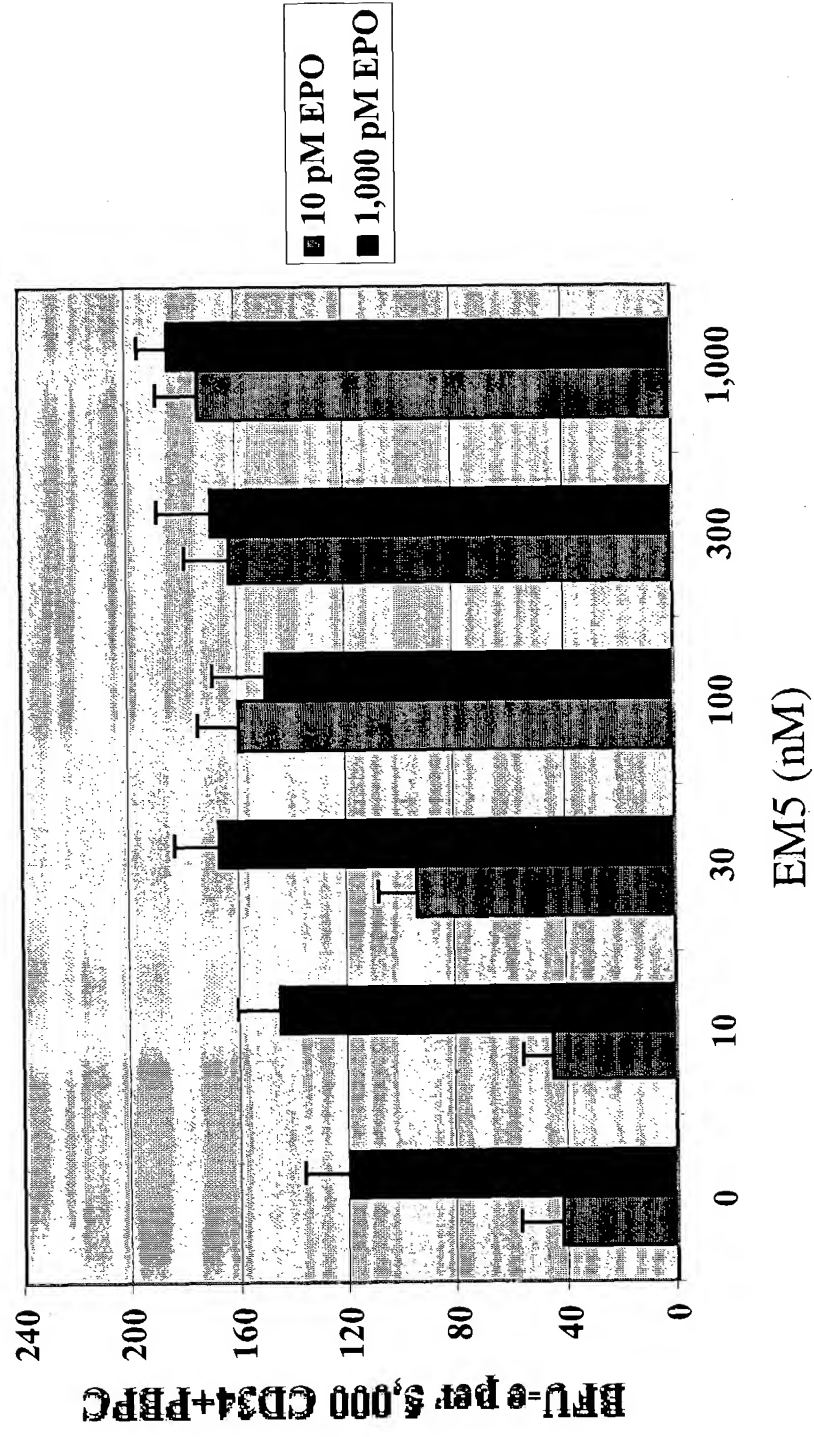
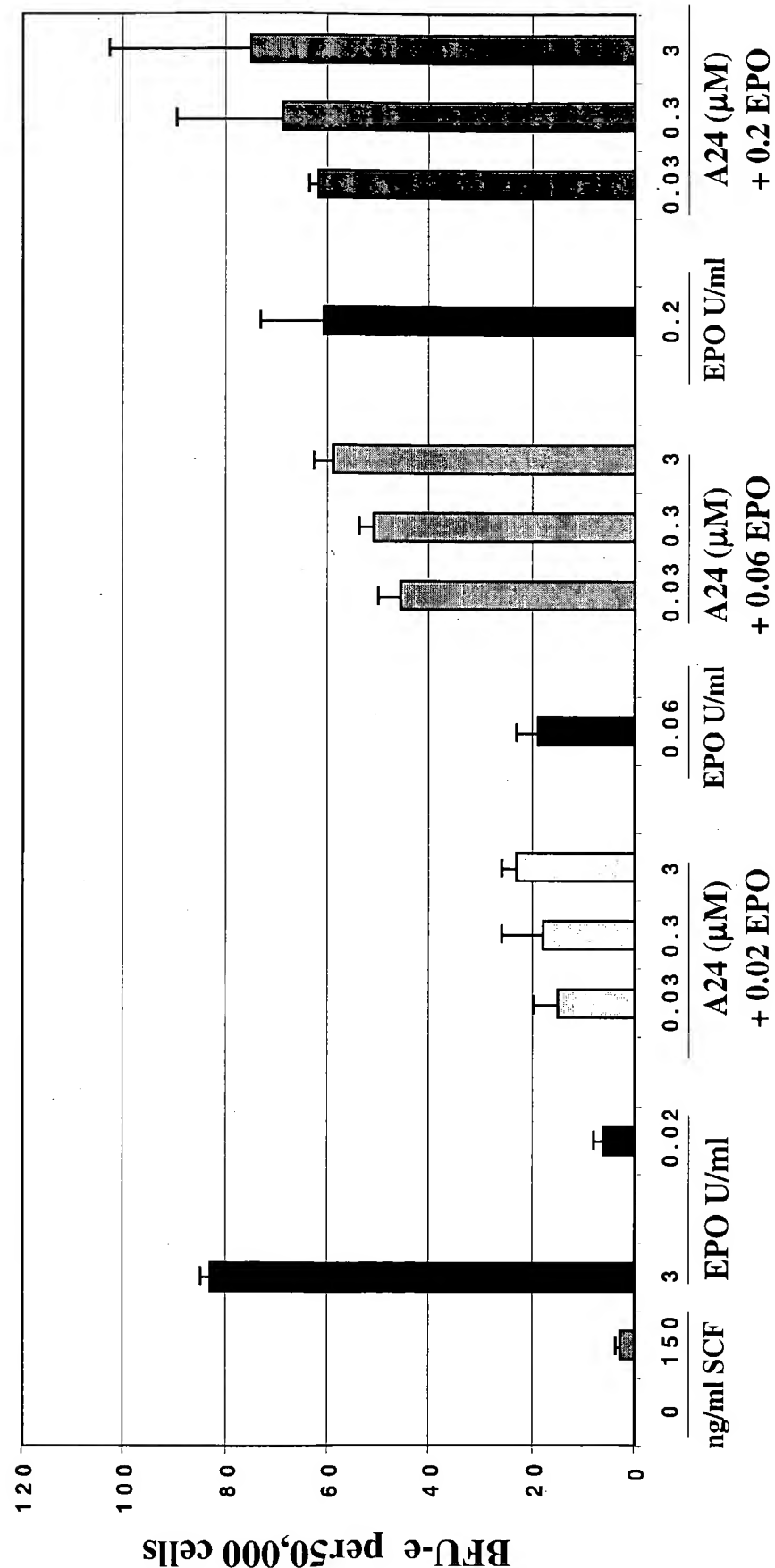


FIGURE 8 -- Synergy between E5A24 small molecule and EPO on erythroid colony formation  
Human bone marrow (day 14)



# FIGURE 9

EFFECT OF EPO-LIKE  
ACTIVITY, SMALL  
MOLECULE - ON  
HEMATOCRIT IN 8  
WEEK OLD C57BL  
MALE MICE

Dose-response

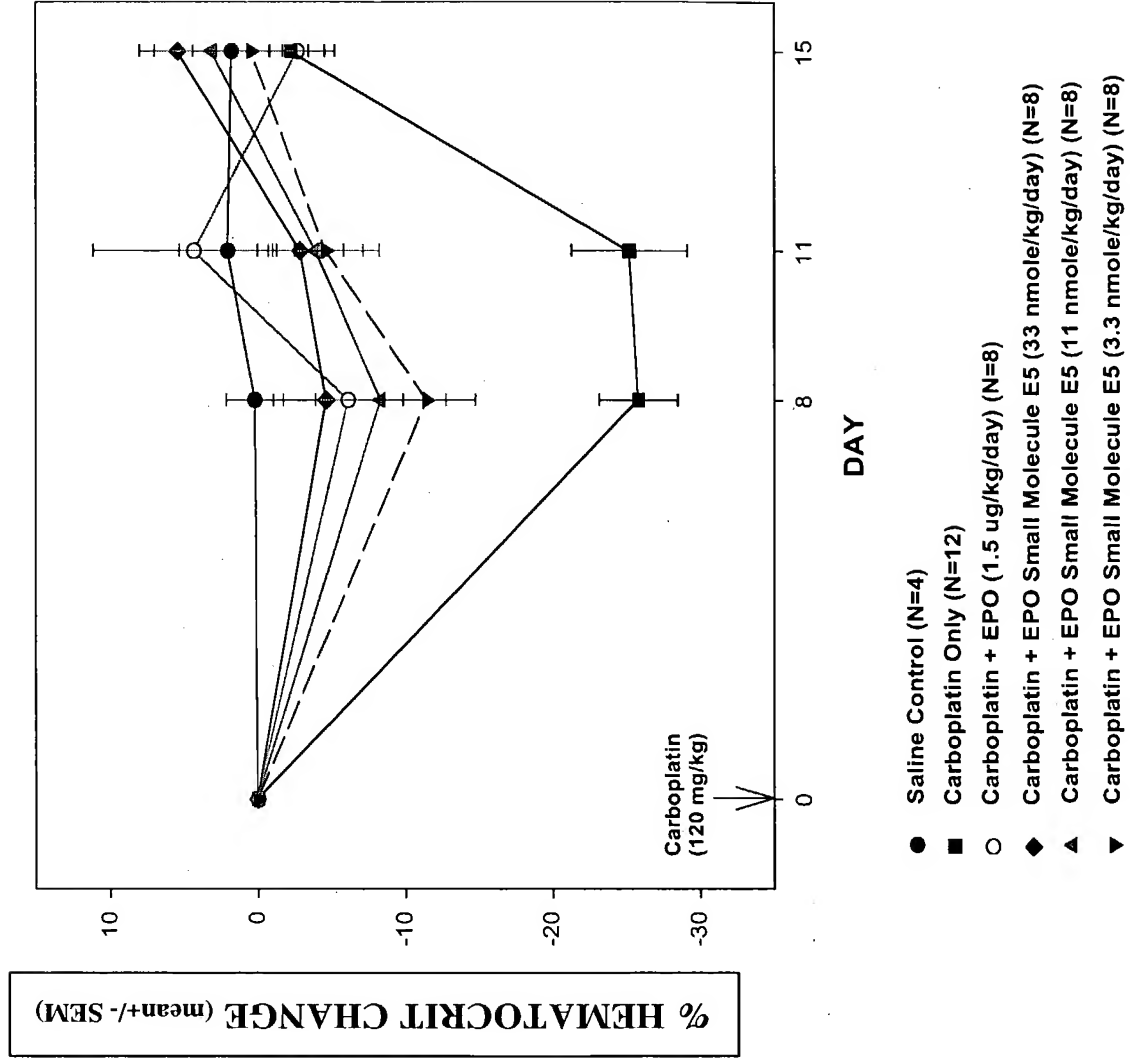
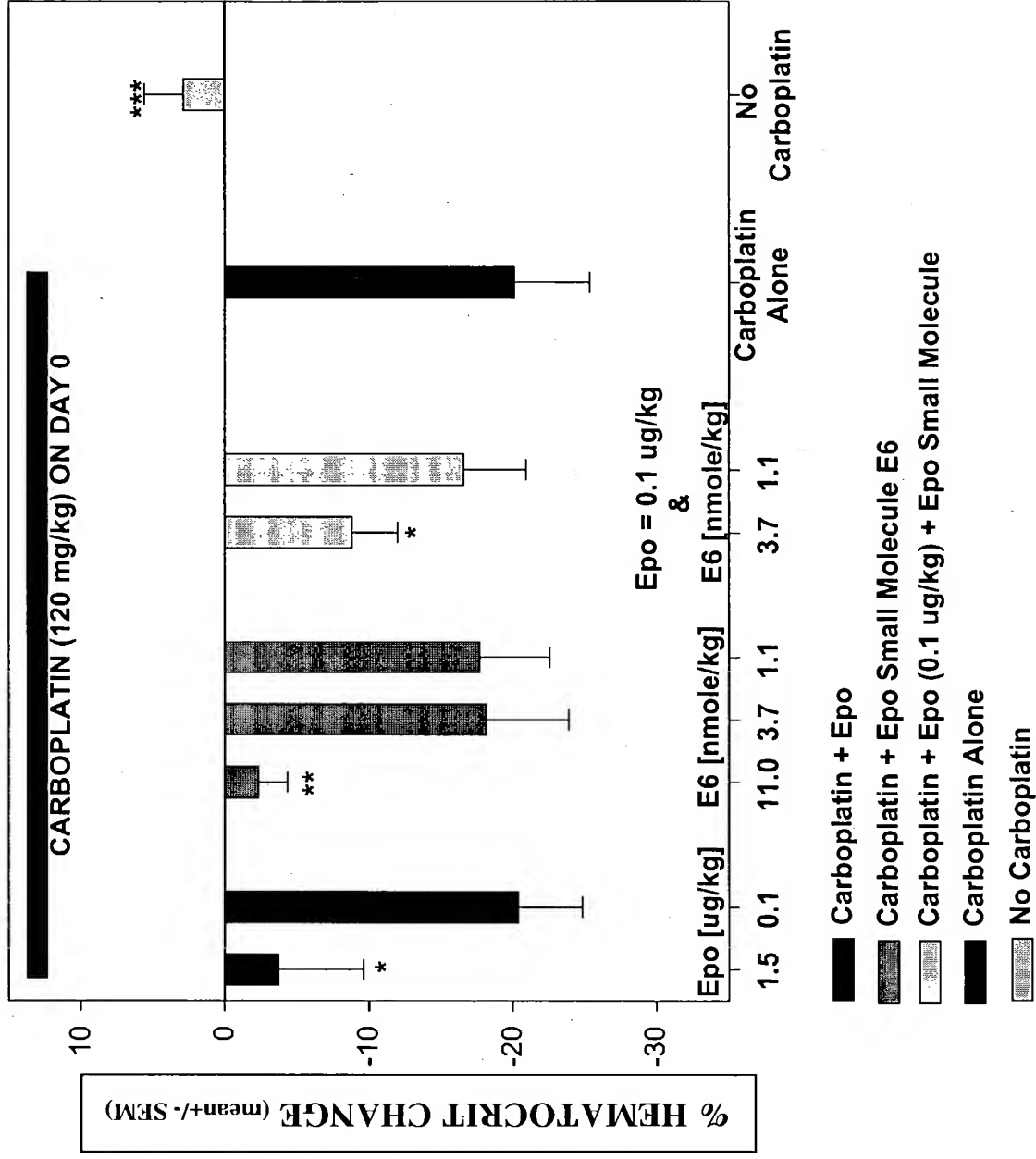


FIGURE 10

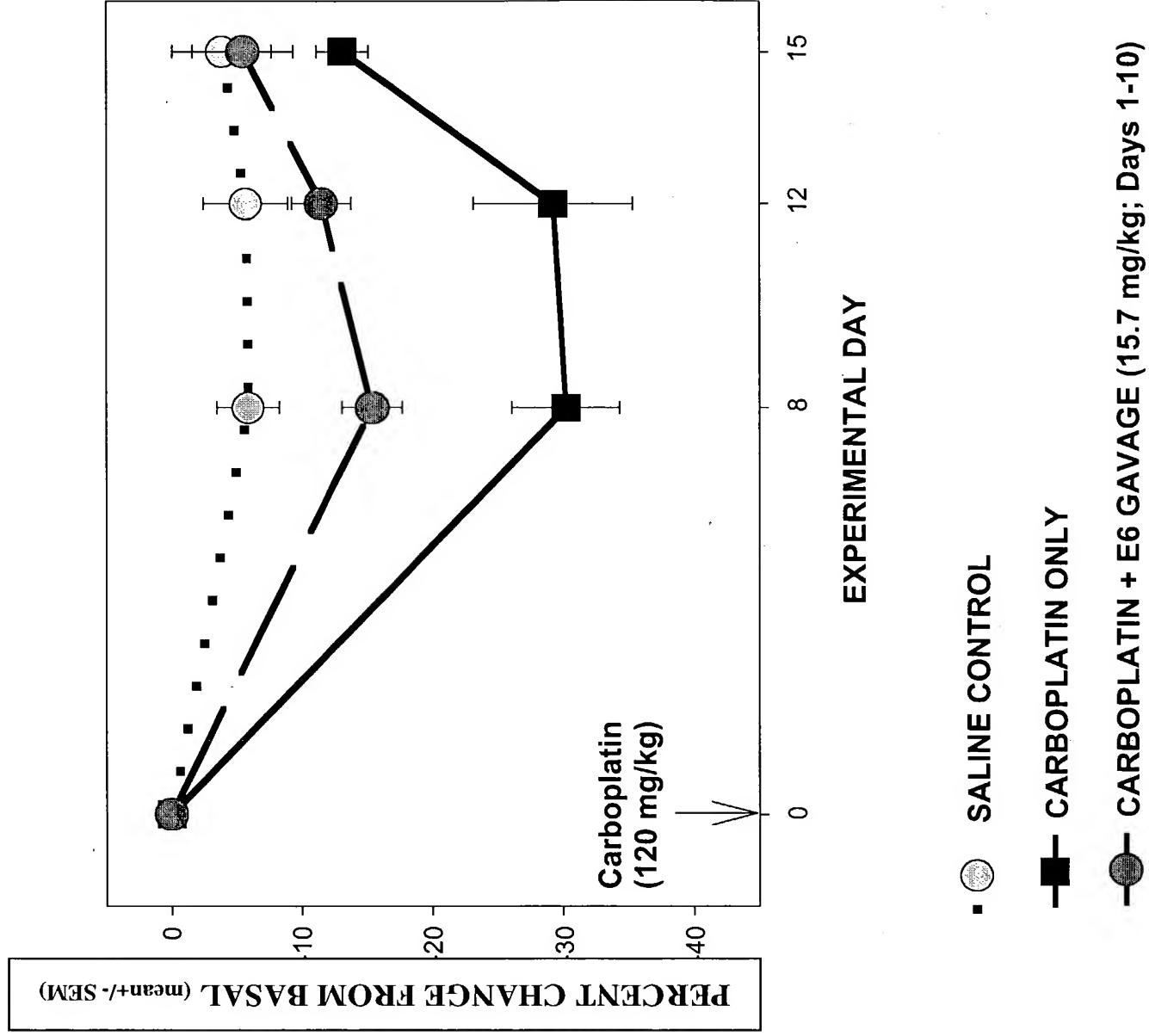
SYNERGISTIC  
EFFECT ON  
CARBOPLATIN-  
INDUCED ANEMIA BY  
ERYTHROPOIETIN  
WITH EPO SMALL  
MOLECULE E6 IN 8  
WEEK OLD MALE  
C57BL MICE  
DAY 10



\* p<0.05 Versus Carboplatin Alone  
\*\* p<0.01 Versus Carboplatin Alone  
\*\*\* p<0.005 Versus Carboplatin Alone

**FIGURE 11**

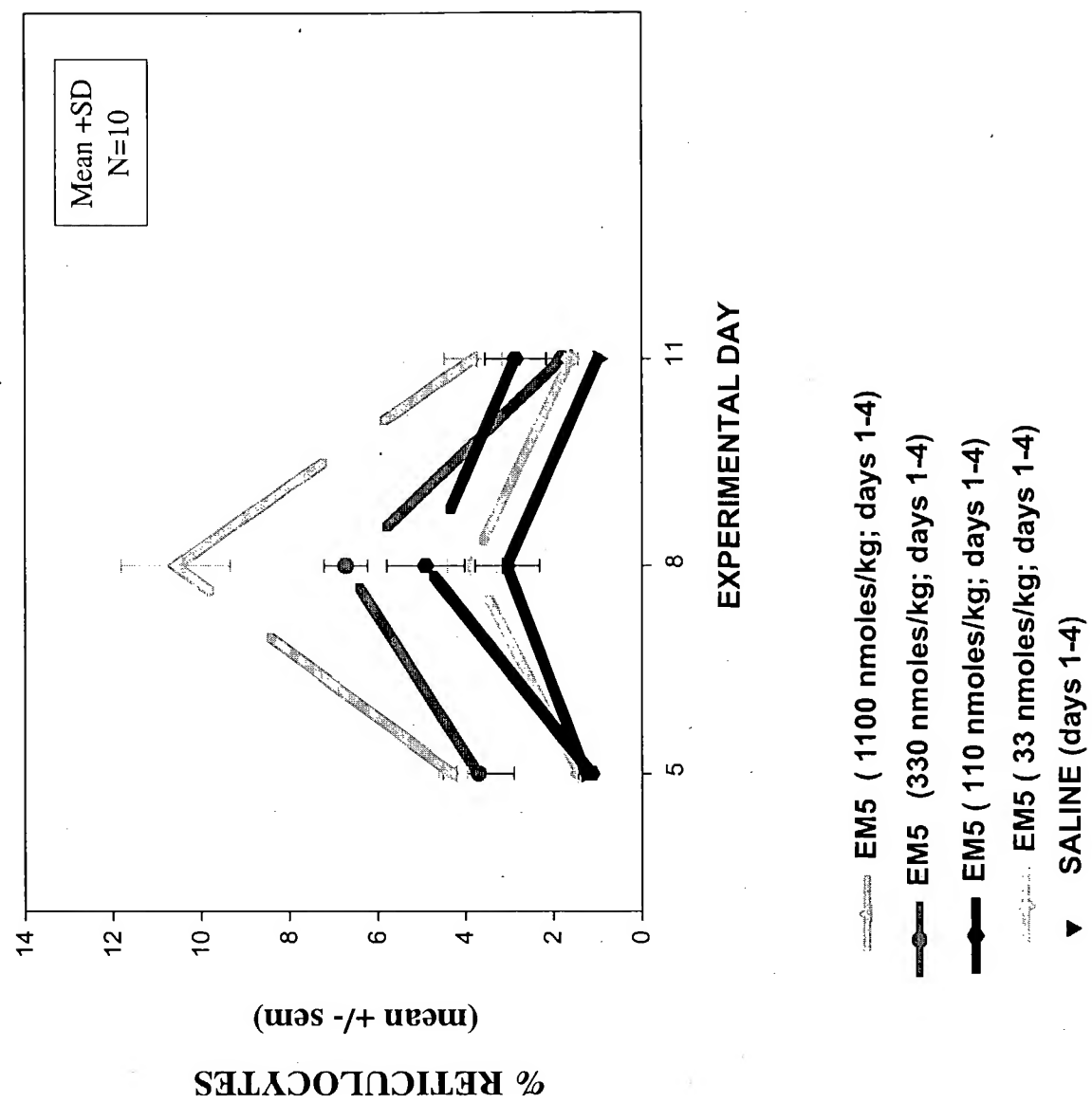
**EFFECT OF E6  
ADMINISTERED  
BY GAVAGE ON  
HEMATOCRIT IN 8  
WEEK-OLD MALE  
C57BL/J MICE**



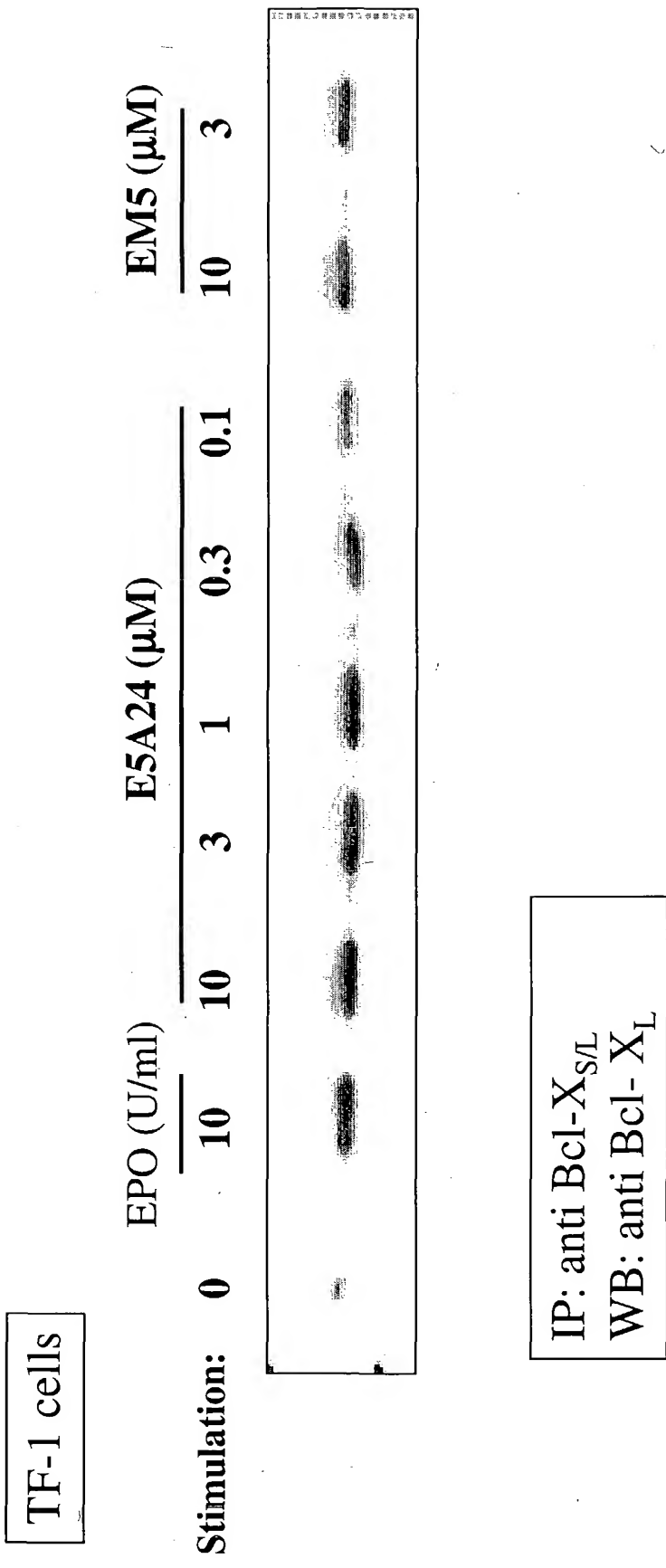
71/71

FIGURE 12

Effect of EPO -  
small molecule  
on reticulocyte  
levels in normal  
animals

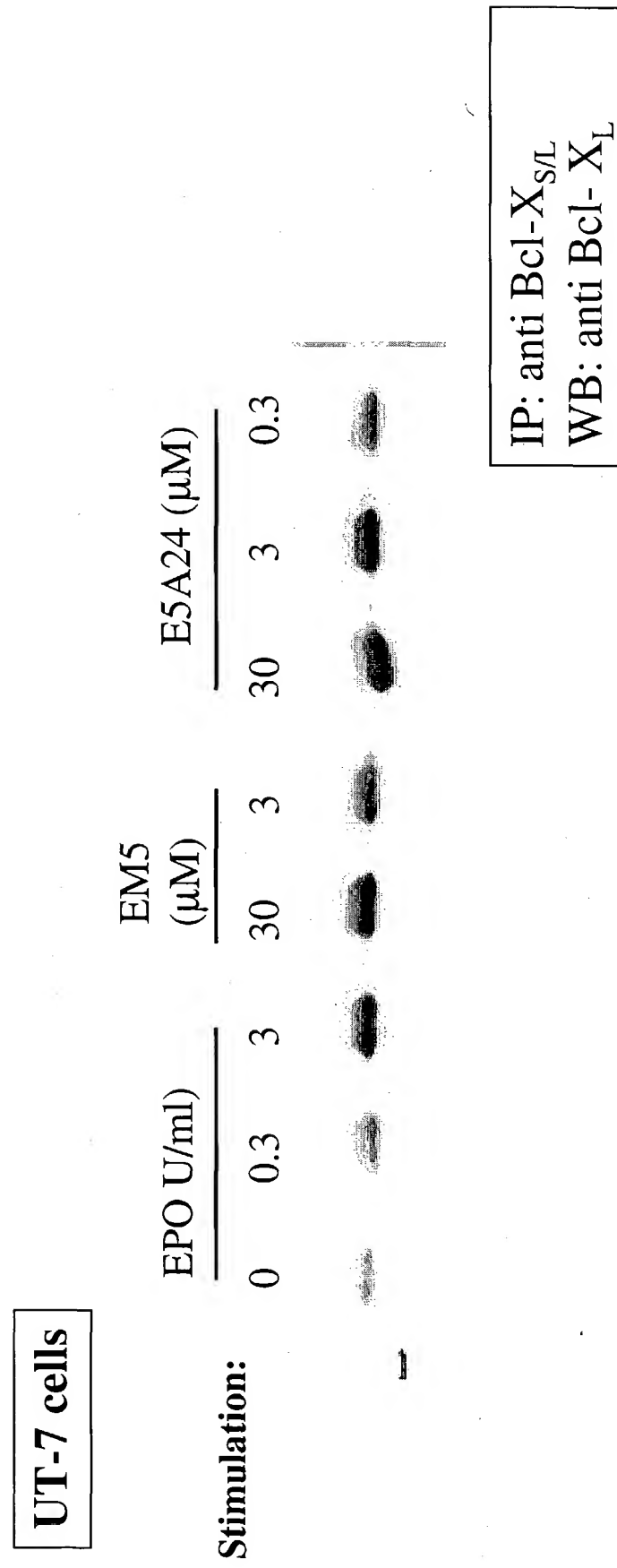


# FIGURE 13 --Small molecule activates Bcl-X<sub>L</sub> expression



LI/C1

FIGURE 14--Small molecules activate Bcl-X<sub>L</sub>  
expression



# FIGURE 15 --Effect of small molecules on P19 cells

- P-19 is a neural-like embryonal carcinoma cell line that undergoes apoptosis upon withdrawal of serum

## Protocol:

- \* Treatment with small molecule or EPO 24 hrs prior to serum withdrawal
- \* Serum withdrawal for 48 hours + EPO or small molecule

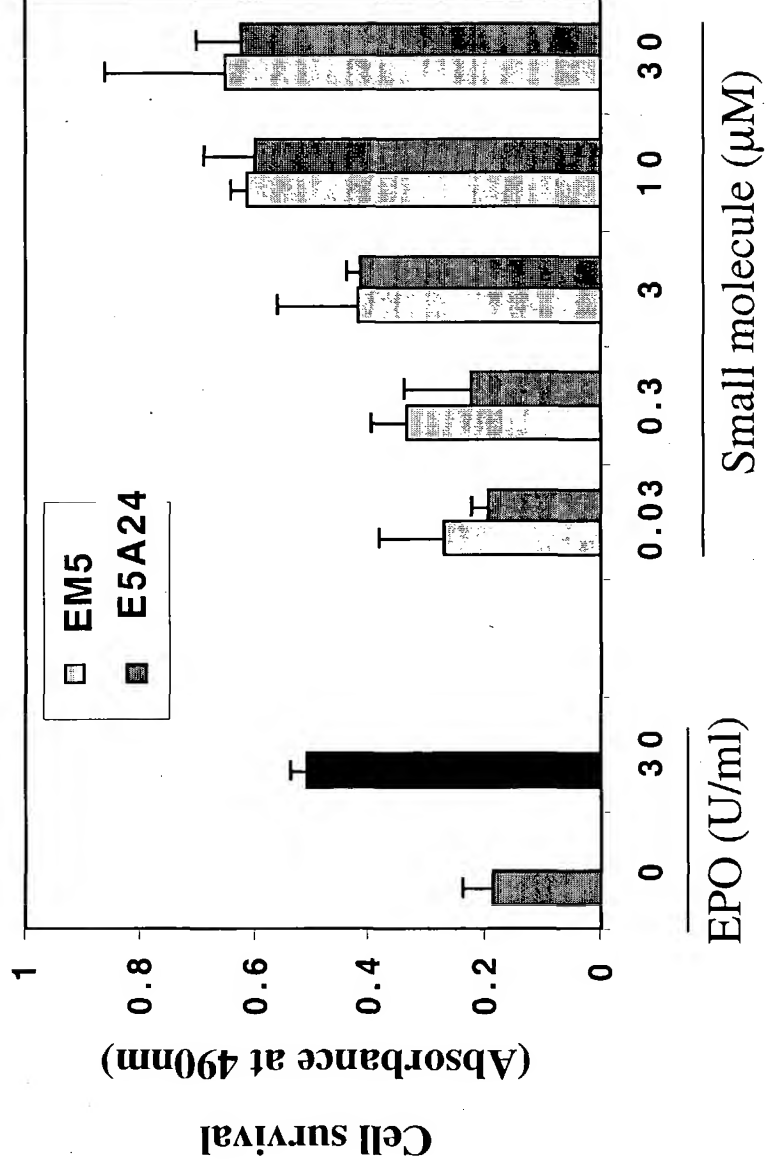
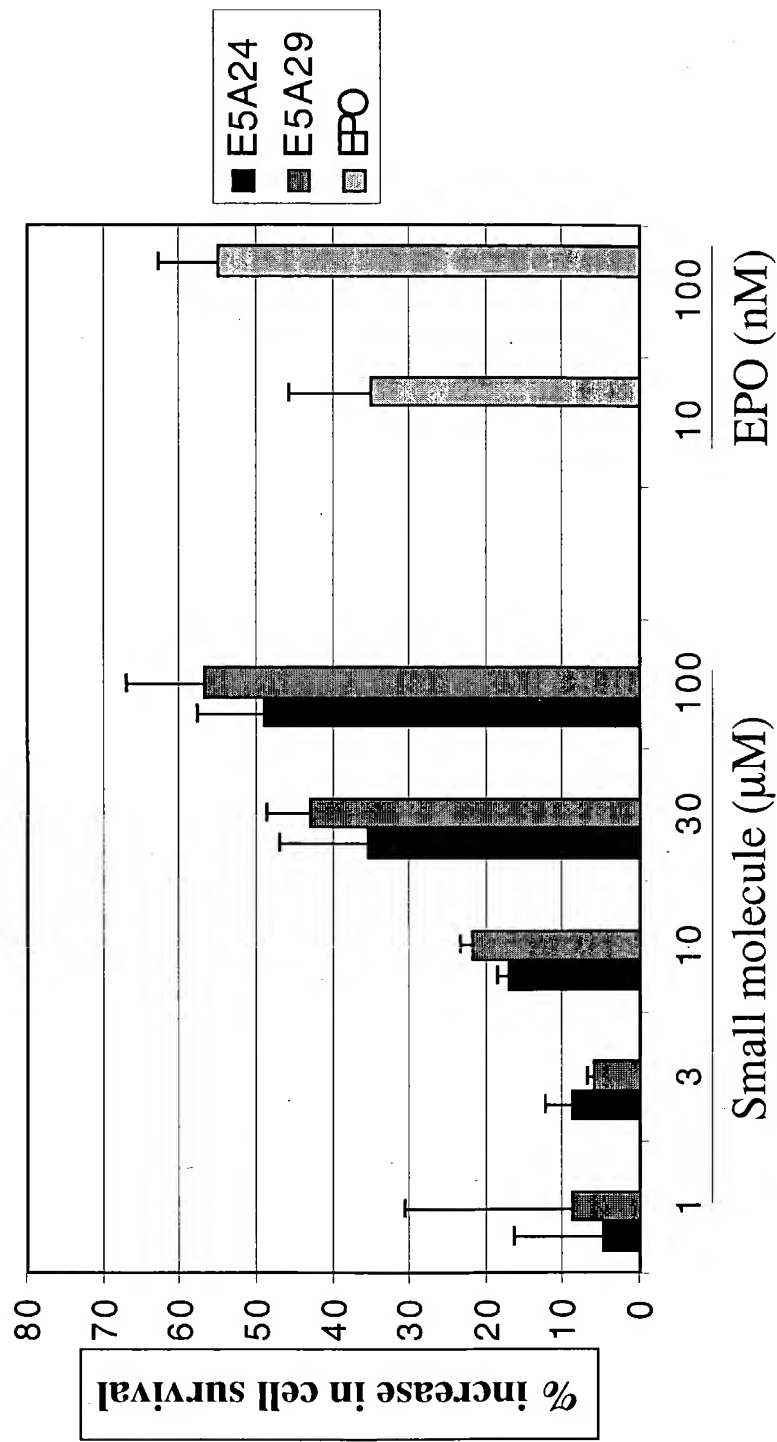


FIGURE 16 --Small molecules prevent neuronal apoptosis  
after glutamate challenge



\* Cortical Neurons isolated from Embryonic day 18 rats  
 \* Treatment with small molecule or EPO for 24 hrs  
 \* Challenge with 300 μM Glutamate for 24 hrs

L1/a1

## FIGURE 17 --Characteristics of EPO-like activity small molecules

- > Bind to EPO-R and Activate EPO-R signaling pathway in the presence and absence of hormone
  - Bind to different site than hormone and do not interfere with EPO binding
- > Act on early erythroid progenitors
  - CFU-e/BFU-e formation in fetal liver cells
  - Synergistic effect with EPO in Bone marrow cells (mouse and human)
- > Promote EPO like anti-apoptotic activity
  - Expression of Bcl-X<sub>L</sub> protein
  - Increase in neuronal survival during glutamate challenge
- > Restore hematocrit level in *in vivo* animal model, given I.P. and orally
  - Act in synergy with EPO
- > Increase reticulocyte levels in normal animals